Zika Virus Induced Neurotropic Brain Injury: Lessons for the Study of Disease Etiology and Vaccine Development Against Pathogens

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Abstract

Studies have found convincing evidence of association of ZIKV with Guillan-Barre Syndrome (GBS), a small preliminary report suggests association with microcephaly in one location. We have previously found a plausible cause (molecular mimicry to Polio Virus Receptors A, B and C). We also found plausible evidence of pathways from ZIKV infection to microcephaly though P53-BAX-mitochondrial mediated apoptosis via the ZIKV capsid protein and the Helicase domain within the NS3 protein. A study of ZIKV infection in neural precursor cells found that infection hampered growth rates (Tang et al., 2016).

To date, no specific mechanism of gestational microcephaly has been demonstrated. Here, we expand the list of plausible mechanisms of microcephaly in ZIKV. In fact, ZIKV appears to be a potential "perfect storm" candidate for microcephaly.

We conclude with open questions and future directions.

Introduction

Zika virus (ZIKV) is a member of the Flaviviridae family, which includes West Nile Virus, St. Louis encephalitis virus, Kunjin virus, yellow fever virus, Dengue virus, and Japanese encephalitis virus. Cellular apoptosis (cell death) and necrosis follow infection for many of these viruses, and appears to be dependent upon several factors, such as viral load, host factors, and specific viral protein induced apoptosis/necrosis pathways, many of which have yet to be fully defined.

P53-BAX-mt Apoptosis and Necrosis

We have previously identified a plausible pathway from ZIKV infection to microcephaly via P53-BAX/mt induced apoptosis triggered by the ZIKV capsid protein (Lyons-Weiler et al.). West Nile Virus (WNV) infections cause severe clinical manifestations including chorioretinitis, acute flaccid paralysis syndrome and fatal meningoencephalitis. Both necrosis and apoptosis are observed morphologically. In West Nile Virus (WNV) infections, viral load with a high infectious dose (multiplicity of infection (m.o.i) > 10), necrosis was observed to be the predominant form of cell death. Apoptosis was observed when the infectious dose was at a low m.o.i. of < 1 [1]. That observation may suggest separate, distinct pathways.

The neuroinvasive WNV capsid protein has been identified to result in apoptosis via HDM2 (E3 ubiquitin-protein ligase MDM2_Q00987) sequestration to the nucleolus. Specifically, HDM2 binds p53 and targets it for degradation at the proteasome. Inhibition of the HDM2-p53 complex via the WNV capsid protein leads to stabilization of p53 and apoptosis through the Bax-mt pathway. Stressed conditions prevent HDM2-mediated p53 degradation and result in p53 activation, inducing apoptosis [2].

The C-terminus of WNV-Cp has been shown to mediate cytotoxic effects on cells [2, 3] through HDM2, an ubiquitin protein ligase that suppresses the transcriptional activity of the tumor suppressor p53 and promotes its degradation. Yang (2008) [3] also demonstrated WNVCp was found to stabilize HDM2 and prevent its degradation, independent of ADP-ribosylation factor (ARF). p14(Arf) also stabilizes p53 by binding to Hdm2 and inhibits the ubiquitination and subsequent proteasome-dependent degradation of p53 (21, 22]. It appeared the C-terminus of WNVCp is responsible for its cytotoxic effects via its interaction with HDM2 [23, 24, 26]. P53 is a major activator in the BAX-mt apoptotic pathway. The mutant (deletion of 106-123) proved unable to induce the translocalization of HDM2 into the nucleolus. This mutant proved consistently unable to bind to HDM2, and also proved incapable of inducing p53 and BAX, which suggests that the C-terminus is responsible for WNV-Cp's cytotoxic effects. The WNV-Cp has been shown to block the binding of HDM2 to p53 via phosphorylation by protein kinase C (PKC) at amino acid residues residing near Ser-83 or within Ser-99 to Ser/Thr-100 and subsequent nucleolar sequestration of HDM2, preventing its interaction with p53. P53 is then free to activate BAX, leading to mitochondrial permeabilization and apoptosis, releasing cytochrome c into the cytosol. West Nile virus capsid protein interaction with importin and HDM2 protein is regulated by protein kinase C-mediated phosphorylation. [4].
Rubella Virus (RV) capsid protein domain, known to cause microcephaly, have also been demonstrated to induce BAX-mediated apoptosis via upregulation of p53 [5, 6].

Apoptosis regulator BAX protein is reported to interact with, and increase the opening of, the mitochondrial voltage-dependent anion channel (VDAC), which leads to the loss in membrane potential and the release of cytochrome c. BAX mediated apoptosis and necrosis occurs by binding to, and antagonizing the apoptosis repressor BCL2. BAX also is known to effect cerebral cortex development (GO0021987) and its related term Microcephalin (IPR022047) [7]. Microcephalin injury by viral protein or molecular mimicry has yet to be reported but needs further consideration.

The apoptotic mechanism of apoptosis necrosis along with observed histopathologic changes is worthy of consideration. The expression of BAX is regulated by the tumor suppressor p53. The majority of BAX is found in the cytosol, but upon initiation of the apoptotic signaling, BAX undergoes a conformational shift and becomes mitochondrial membrane associated. Organelle disruption, therefore, is a characteristic of BAX-mt pathway apoptosis (Figure 1).

Figure 1. Proposed pathway of the Zika virus capsid protein (ZIKVCp) induction of p53-BAX mediated mitochondrial permeabilization with release of cytochrome c

The C-terminal 16 residues of the West Nile Virus capsid protein (WNV-Cp-orange) binds the E3 ligase HDM2 (MDM2-green) and chaperone it to the nucleolus which prevents HDM2 binding to p53. Since p53 is degraded in the presence of HDM2 (MDM2), the sequestration of HDM2 by WNVCP to the nucleolus prevents that interaction, stabilizing p53. The stabilization of p53 allows for BAX activation and subsequent apoptosis.

This result in the release of cytochrome c and other pro-apoptotic factors from the mitochondria is often referred to as mitochondrial outer membrane permeabilization. Through necrosis and organelle disruption, the subsequent release of cytochrome c from the disrupted mitochondria leads to activation of caspases. This defines a direct role for BAX in mitochondrial outer membrane permeabilization and necrosis with organelle disruption and necrosis [8].

**Caspase-9 Apoptosis**

Apoptosis can occur through distinct caspase-independent and -dependent pathways [9]. Yang et al in 2002 also reported that the WNV capsid protein (WNV-Cp) drives apoptosis in vitro through the mitochondrial/caspase-9 pathway and subsequent caspase-3 activation in the brain [10]. Samuel et al in 2007, reported their findings that subsequent to infection, WNV induced Caspase-3 activation and apoptosis in the brain [11]. Inhibition of caspases in WNV infection has been shown to limit apoptosis [12].

Caspase-9 is activated by the mitochondrial release of cytochrome c into the cytosol (Zou et al., 1999) and initiates the induction of Caspase-3. Caspase-3 induces cells to undergo characteristic morphological changes in caspase-independent apoptosis (Table 1):

1. Formation of apoptotic bodies consisting of cytoplasm with tightly packed organelles with or without a nuclear fragment
2. Organelle integrity is still maintained and remain enclosed within an intact plasma membrane

<table>
<thead>
<tr>
<th>Table 1. Comparative Histopathologic Characteristics of Necrosis and Apoptosis</th>
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<tbody>
<tr>
<td><strong>Apoptosis</strong></td>
</tr>
<tr>
<td>Single cells or small clusters of cells</td>
</tr>
<tr>
<td>Cell shrinkage and convolution</td>
</tr>
<tr>
<td>Pyknosis and karyorrhexis</td>
</tr>
<tr>
<td>Intact cell membrane</td>
</tr>
<tr>
<td>Cytoplasm retains and apoptotic bodies</td>
</tr>
<tr>
<td>No inflammation</td>
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</tbody>
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**Figure 2. Caspase apoptosis.**

The apoptotic body is indicated by the arrow enclosed by cytoplasm. The mitochondria organelle is intact in contrast to necrosis through BAX induction [Figure from, 13, reproduced with permission].

**Caspase-8** does not appear to be involved in the WNV-Cp induced apoptosis. Deletion of the C-terminal residues of WNV-Cp reduced the induction of caspase-9 and subsequent apoptosis and thereby defining the caspases-9 induction domain as residing between residues 67-122 in the capsid protein domain. In contrast to the BAX pathway, the caspase-9 pathway does not appear to destabilize the mitochondrial membrane and subsequent mitochondrial outer membrane permeabilization as observed through the p53-BAX pathway.

**Viral NS3 and Caspase Apoptosis**

Ramanathan et al demonstrated that the viral protein NS3 alone was sufficient to induce caspase-8 apoptosis. Two primary domains within NS3 were identified. Expressions of the protease and helicase domains were sufficient to trigger apoptosis in WNV infections [14].

Chu et al in 2003 [1] found that after cytochrome c was released during initial apoptosis, caspases-9 was activated increasing caspase-3 leading to further apoptosis. This would suggest the p53-BAX pathway
leads to later caspase-initiated apoptosis via release of cytochrome c from the mitochondria. This is required for the formation of the apoptosome, which, in turn, is necessary for activation of pro-caspase-9. This would imply both the p53-BAX pathway via the viral capsid protein and caspase-9 activation pathways via the NS3 Peptidase 7 and the C-terminal Helicase (HELICc) domains are involved in the necrosis and apoptosis.

Proposed Mechanisms of Apoptosis/ Necrosis in Zika Virus Infection

The histomorphologic changes in the brain resulting from ZIKV-infection have recently been described [15], and the variance from typical development trajectories have be characterized (Brasil et al., 2016) The authors reported on a case of an expectant mother who had a febrile illness with rash at the end of the first trimester of pregnancy while she was living in Brazil.

Numerous histomorphologic findings of both apoptosis and necrosis were identified. Gross findings included intracranial cortical and subcortical calcifications, lissencephaly, and autolysis. Electron microscopy of the brain identified ruptured and lysed neuronal cells in association with numerous icosahedral, virus-like particles. The presence of ZIKV was confirmed by PCR. The large number of viral particles associated with the necrosis and apoptosis is similar to the previously described association of primary necrosis at high m.o.i >10 and apoptosis when the multiplicity of infection is low (m.o.i. < 1). Known infectious causes of intracranial calcifications include toxoplasmosis, rubella, Cytomegalovirus (CMV) and Herpes simplex virus (HSV). The mechanism of placental transfer is not known, and we suspect one or more co-factors may explain the restricted geography of microcephaly in areas with sustained local transmission of Zika virus. 

Figure 3. Electron microscopy from ZIKV confirmed microcephaly [15]; Figure used with permission

Zika virus particles were identified post-mortem in the brain (arrow).

Results

EVIDENCE FROM HOMOLOGY

Capsid Protein Domain

The ZIKV capsid domain (1-122; Figure 4 and 5) was first investigated for homology with neuroinvasive WNV (WNVnv) and Dengue virus representative sequences (DENV1, DENV2, DENV3, AND DENV4). The N-terminal residues demonstrated the greatest homology, particularly from Gly-40-Pro-61. The C-terminal residues previously demonstrated to be involved in Bax mediated apoptosis from WNV-Cp (67-100) were found to have 42% identity (Figure 5). The p53-HDM2 binding residues which were reported to be phosphorylated and flank the p53-HDM2 binding domain (Ser-83 and Ser-100) were not found in ZIKV or DENV. In ZIKV and DENV, these serine residues were replaced by Lys-83 in ZIKV and Gly-83 in DENV1, DENV2, DENV3, and DENV4. The C-terminal WNVnv Ser-100 residue was replaced by Lys-100 in both ZIKV and The Dengue virus sequences. However, the serine residues (Ser-83 and Ser-100) were flanked by highly conserved, positively charged residues (FKK) immediately from positions 84-86 and from 97-100. These electrostatic interactions may also play a role in BAX mediated injury.

Figure 4. HDM2 Binding Domain in the Flavivirus Capsid Protein

The HDM2 critical binding domain is identified by the red bar (83-100) and the C-terminal 18 residues identified by Yang et al (2008) are located below the green bar (105-123). Note the Serine residues at position 83 and 100 from WNVnv are flanked by conserved, positively charged Arginine and Lysine residues in all sequences. The conserved Lysine residues (101, 103, and 104) within the C-terminal 18 residues in West Nile Virus known to bind to MKRN1 are enclosed in the red rectangle. In Zika virus, Lys-101 and Lys-104 are replaced by Arg-101 and Arg-103 in Zika virus which may affect binding and ZIKVCp ubiquination by MKRN1. The amino acid alignment performed with the CLUSTAL Omega algorithm and visualized with Jalview.

Figure 5. Multiple Sequence Alignment of the Flavivirus Capsid Domain

A significant difference is that ZIKV has highest hydrophobicity profile within the C-terminus of the capsid protein domain (Figure 6). Those residues are flanked by positively charged Lysine and Arginine residues that may contribute to interactions.

Figure 6. Hydrophobicity of the capsid domain of ZIKV, WNV, and DENV.

The hydrophobicity is generally conserved with the exception of region 89-96 within the C-terminus of the ZIKVCp as compared to West Nile Virus and Dengue virus (DENV1, DENV2, DENV3, and DENV4). The greater hydrophobicity demonstrated within that region of ZIKV compared to West Nile Virus and Dengue virus may enhance binding to HDM2.
Structural Homology

The peptide secondary structures of WNVCp and ZIKVCp were generated with PHYRE2 [27]. The WNVCp was found to consist of 6 helical domains (10-24, 27-38, 43-57, 63-71, 74-105, and 98-121) which represented 79% of the WNVCp. 27% was predicted to be disordered (FIGURE 7). Helical domains 5 and 6 represent the HDM2 binding region (Yang, 2002 and 2008).

FIGURE 7. Secondary Structure Prediction of the West Nile Virus Capsid Protein.

The full length WNVCp amino acid sequence (accession number YP_005097850.1) secondary structure was predicted using PHYRE2. 79% of the peptide is predicted to be in helical domains with 27% predicted to be disordered and located between the helical domains. 59% of the residues were modelled at >90% confidence.

The WNVCp peptide sequence, accession number YP_005097850.1, was aligned with PDB|1SFK Core (C) protein from West Nile Virus, subtype Kunjin using Clustal Omega and saved as a FASTA file. The alignment was modeled using Modeller in UCSF Chimera. The conserved residues within the ZIKVCp C-terminus (position 83-100) were located at Phe-83, Lys-84, Lys-85, Leu-87, Ile-94, Asn-95 and Arg-98. Residues 99-122 could not be modeled as there was no available reliable template. The RMSD from the region 83-98 was calculated to be 33.669.

FIGURE 8. Homology modelling of the WNVCp C-terminal HDM2 binding Domain.

Interactive modelling was performed using Modeller with UCSF Chimera. The template, 1SFK Core (C) protein from West Nile Virus, subtype Kunjin, ribbon is colored tan on the left. The model on the right (purple ribbon) is the WNVCp, accession YP_005097850.1. In both models, the conserved residues with ZIKVCp (Phe-83, Lys-84, Lys-85, Leu-87, Ile-94, Asn-95 and Arg-98) are colored red. Sequence identity was calculated to be 94.59% for the entire model (Leu-25-Arg-98). Residues 99-123 could not be modelled with accuracy due to a lack of a template that included the terminal 24 residues.

Similarly, the full length ZIKVCp peptide sequence, ZIKACp|BRAZIL, was submitted through the Protein Model Portal for interactive modelling. Templates included 1R6R|A (Chain A, Solution Structure Of Dengue Virus Capsid Protein Reveals A New Fold-39% identity), ISFK|A (Chain A, Core (c) Protein From West Nile Virus, Subtype Kunjin-49%), 4LQ0|A (Chain A, Structure Of Cbm32-3 From A Family 31 Glycoside Hydrolase From Clostridium Perfringens-34%), and WWJ3|A (Chain A, Crystal Structure Of The Asparagine Transamidosome From Pseudomonas Aeruginosa28%). The selected modelling template were 1R6R|Chain A and ISFK|Chain A. The secondary structure prediction was obtained through PHYRE2 (Figure 9).

Figure 9. Secondary structure of the Zika Virus Capsid Protein

The full length ZIKVCp amino acid sequence (BRAZIL) secondary structure was predicted using PHYRE2. 81% of the peptide is predicted to be in helical domains with 26% predicted to be disordered and located between the helical domains. The ZIKVCp was predicted to have 5 helical domains as the WNV H5 and H6 domain are combined in the ZIKVCp. In WNVCp, the H5 and H6 domains are separated by 2 Glycine residues. This region was predicted to be disordered.

Comparative interactive modelling was then completed in UCSF Chimera, aligning the conserved regions between the WNVCp and the ZIKVCp. 59% of residues modelled at >90% confidence. The ZIKVCp alignment with 1SFK [28] ; sequence identity 48.68%) was selected (Figure 10). 64% of the residues were modelled at >90% confidence.

Figure 10. Comparative Model of ZIKVCp and WNVCp

In Panel A, the West Nile Virus capsid protein (WNVCp) using the PDB file 1SFK|ChainA is depicted. The conserved residues within the HDM2 interactome of the C-terminus are identified at Phe-83, Lys-84, Lys-85, Leu-87, Ile-94, Asn-95, and Arg-98 and are colored red. In Panel B, the Zika virus capsid protein (ZIKVCp) model was generated in UCSF Chimera with Modeler using 1SFK|A as the modeling template. The corresponding conserved residues at Phe-84, Lys-85, Lys-86, Leu-88, Ile-95, Asn-96, and Arg-98 are colored red. The 3D structure is highly conserved between WNVCp and ZIKVCp. Residues 99-123 could not be modelled with accuracy due to a lack of a template that contained the terminal 23 residues.

NS3 DOMAIN: PEPTIDASE 7 AND HELICASE HOMOLOGY

The Peptidase and C-terminal Helicase domains were evaluated for homology as these domains were previously discussed as being sufficient for caspase induced apoptosis. Between position 1620-1670 of the Peptidase 7 conserved domain in NS3 (Figure 11), the overall homology was found to have 56% identity, but
also 78% positives suggesting the C-terminus may be the region of shared interaction with caspases.

The Helicase domain from position 1867-1976 (Figure 12) demonstrated 76% homology and 81% positives within the C-terminus. This may suggest a greater role of the Helicase domain in caspase induction and apoptosis.

**Figure 11. Peptidase 7 Caspase Induction Domain within NS3**

**Figure 12. Helicase Domain within NS3**

In addition to these findings, we have previously reported a finding within the South American ZIKV polyprotein sequences resulting in the discovery of a single amino acid change unique to the South American clade (M2633V) within the motif EEP(M/V)LVQ of the NS5 gene. This motif resides in a FtsJ-like methyltransferase conserved domain, and is a S-adenosylmethionine-dependent methyltransferase (SAM or AdoMet-MTase) that is used as a substrate for methyl transfer, creating the product S-adenosyl-L-homocysteine (AdoHcy).

S-Adenosylhomocysteine (SAH) Hydrolase Deficiency is usually caused by a defect in the AHCY gene, which codes for SAH. SAH Deficiency causes accumulation of guanidinoacetate, homocysteine, methionine, s-adenosylhomocysteine and s-adenosylmethionine in plasma, and methionine in spinal fluid. Symptoms of SAH deficiency include cerebral atrophy, dysmorphism, strabismus, jaundice, mental and motor retardation (SMPD, 2016). It is possible that the M2633V mutation in the S Am ZIKV interferes with SAH hydrolysis.

**miRNA Discovery**

Our analyses of the genomic sequences of ZIKV obtained from the publicly available databases in search of plausible specific molecular mechanisms of microcephaly also yielded a finding of an miRNA-like hairpin secondary structure located at the 3’ terminus of the 3’ untranslated region (UTR) in ZIKV [29]. This structure located within the sRNA of ZIKV was found to have considerable structural and sequence homology with a West Nile Virus miRNA in the 3’ UTR. The ZIKV miRNA sequence was submitted as a BLASTn query through MiRBase to search for a miRNA homolog. A stem loop and mature human miRNA homolog, hsa-mir-147a, was identified. 251 predicted human target transcripts included Neurofascin (NFASC), Synaptic Vesicle Glycoprotein 2A (SV2A), and Neurofibromin 1 (NF1) were identified as neurotropic targets [29].

**Discussion**

**Functional Inferences**

1. Both p53-BAX mediated necrosis/apoptosis and caspase-mediated apoptosis appear to be active pathways in Zika virus infection as identified by previously reported histomorphologic features.
2. The viral capsid protein homology indicates the region 83-100 may be responsible for the p53-BAX mediated necrosis/apoptosis observed in ZIKV infection. This is testable via epitope testing in mice or primates.
3. The histomorphology seen in Zika virus encephalopathy is indicative of both a BAX-mt and Caspace pathway of necrosis and apoptosis.
4. Caspase-induced apoptosis via the Peptidase 7 and Helicase domain of NS3 may also be involved in ZIKA Virus apoptosis, as has been previously described with West Nile Virus.
5. Causes of selective neurotropism resulting in microcephaly (e.g., Microcephalin injury) remain to be identified

**OPEN QUESTIONS, AND FUTURE DIRECTIONS**

The histomorphology seen in Zika virus encephalopathy is indicative of both a BAX-mt and Caspace pathway of necrosis and apoptosis. We found significant homology is identified between West Nile Virus, Dengue Virus, and Zika Virus within the C-terminus of the Peptidase-7 and Helicase domains of the NS3 protein, further giving evidence to cytochrome c-> caspase initiated apoptosis in ZIKA Virus infection.

In summary, it would appear that the C-terminus of the ZIKV capsid protein domain (ZIKVCp) may very well be responsible for the BAX apoptosis/necrosis pathway, particularly when the histomorphologic findings are considered. However, without the phosphorylated serine residues at the 3’ and 5’ ends, ZIKV and DENV may not be able to bind HDM2 and may use another mechanism to induce p53, block anti-apoptotic Bcl-2, or bypass BAX and preferentially use caspase induced apoptosis. The histomorphologic evidence of both apoptotic bodies and necrosis in intracranial ZIKV infection would implicate both pathways in ZIKV induced apoptosis/necrosis. Additionally, with the significant homology that exists with the NS3 domain, specifically the C-terminus of both the Peptidase 7 and Helicase domains, evidence suggests a caspase induced apoptosis as a result of cytochrome c release following BAX mitochondrial permeabilization.

A novel miRNA in ZIKV is also a plausible candidate, especially given it targeting of neurotropic transcripts such as neurofascin, synaptic vesicle glycoprotein 2A, neurofibromin 1, SAM and SH3 domain containing 1,
and neurogenin 2 [29].

All of the plausible molecular mechanisms represent a testable hypothesis, and this study, as well as (Lyons-Weiler et al., [17]) provides a blueprint for directions for high-priority research. ZIKV appears to be a “perfect storm” candidate for microcephaly, with at least three independent specific molecular triggers of gestational microcephaly: P5-BAX induced apoptosis, mitochondrial damage induced apoptosis, and predictive ZIKV 3’UTR-encoded miRNA. There may be other candidate mechanisms we have not yet identified.

Open Questions

If ZIKV appears to be a perfect storm candidate,

(1) Why are there no cases of microcephaly due to ZIKV infection during pregnancy outside of Brazil?

(2) What might be the missing co-factors that activate the route(s) to microcephaly in Brazil?

(3) What factors might produce phenomimics of ZIKV induced microcephaly;

(4) How can the medical and psychological consequences (fall-out) of false positive risk of microcephaly be minimized;

(5) What are possible ways to safely prevent microcephaly due to ZIKV infection in Brazil?

(6) What lessons is ZIKV teaching us about our approach to understanding medical conditions with a multitude of plausible causal factors and complex etiologies (autism, autoimmune disorders)?

One of the lessons we are learning from Zika is that public health policy should anticipate, especially with emerging diseases, multiple factors, including both necessary and potentially unknown co-factors, as well as phenomimiccy due to unrelated factors. Studies designed to test for interactions among factors should be par for the course; initial univariate studies are important, however, they may miss associations due to viruses and co-factors. As we have said before, something is different in Brazil.

Another important lesson is that we anticipate that any vaccine against ZIKV that includes the NS5 epitope EEPVLVQ may induce either maternal antibodies or autoimmunity against a protein that is critical for proper brain formation, especially if the vaccine is adjuvanted. We strongly recommend only the use of non-adjuvanted virus-like particles with safe epitopes screened for high local homology for ZIKV vaccines to avoid vaccine-induced brain injuries, and for all vaccines to avoid the induction of autoimmunity and autoimmune disorders [16].

Future Directions

Our analyses point to multiple, specific, testable hypotheses for ZIKV-induced microcephaly. However, at least nine other hypotheses remain to be tested (Lyons-Weiler et al., 2016). Among these include interactions among factors, and suspicion of one or more co-factors required for Zika-induced microcephaly due to (a) the geographic restriction of microcephaly associated with gestational Zika virus infection, and (b) the absence of any amino acid change in Zika proteins unique to viruses shared by microcephaly cases [30]. With our analyses, and the findings of Mlakar et al. [32], Tang et al., [20]; Lyons-Weiler et al., [16], the plausibility of ZIKV-induced microcephaly is increasing. The mystery of the absence of microcephaly in babies born to mothers with ZIKV infections in pregnancies not associated with gestational time in Brazil remains.

Surprisingly large amount of molecular mimicry between epitopes in Zika virus proteins and human neurological proteins (Lucchese and Kanduc, 2016)[31] suggestion that a great deal of caution is warranted for vaccine development for Zika virus. A strategy that excludes peptides with high homology with human proteins is strongly recommended to avoid potential complications resulting from molecular mimicry.

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Illustrations

Illustration 1

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Figure 2. Caspace apoptosis. The apoptotic body is indicated by the arrow enclosed by cytoplasm. The mitochondria organelle is intact in contrast to necrosis through BAX induction [Figure from, 13, reproduced with permission].
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Illustration 4

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